REMARKS

I. Status of the Application

Claims 1-8 were originally filed in this application. Applicants amend claim 1 to recite that "the pharmaceutical composition attenuates the effect of pathogenic bacterial enterotoxins." This amendment is supported throughout the application, for example, at page 12, lines 11-19; page 14, lines 12-18; and at pages 24-37, Examples 2-7, discussed further below. Applicants add new claim 9 to recite that the composition also "attenuates transepithelial migration of polymorphonuclear leukocytes." Claim 9 is also supported throughout the application, for example, at 17, lines 10-16, and pages 38-46, Example 8.

Applicants cancel claims 5 and 6, without prejudice or disclaimer, and replace those claims with new claims 10-14. Like claim 1, claim 10 recites a method of administering the pharmaceutical composition of claim 1 such that the pharmaceutical composition is in an amount "effective to attenuate the effect of pathogenic bacterial enterotoxins." As with claim 9, claim 14 recites a method such that the composition is also "effective to attenuate transepithelial migration of polymorphonuclear leukocytes." Claims 10 and 14 are supported by the application as a whole, for example, as for claims 1 and 9. New method claims 11-13 parallel composition claims 2-4, and are thus supported by the claims as originally filed.

Applicants amend claim 4 to remove a few of the pathogenic bacterial species listed in that claim. Finally, Applicants amend claims 7 and 8 to alter their language in accordance with claim 10. In claim 7, "mammal" is replaced with "host." The word

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"host" is supported by the application as a whole. Specific support may be found, for example, at page 9, lines 8-15.

II. Objection to the Specification

The Office objects to the specification, asserting that it does not provide sufficient antecedent basis for "putrescine" in claim 3. However, "putrescine" is recited and discussed at page 13, line 10, of the specification. The originally filed claims are also a part of the application's disclosure. 35 U.S.C. § 112. Thus, Applicants respectfully request the withdrawal of this objection.

III. Rejection of Claims 1-8 under 35 U.S.C. § 112, First Paragraph

The Office rejected claims 1-8, asserting that they are not enabled. (Office Action at pages 2-6.) Applicants traverse this rejection, but note that the claims, as amended, recite a pharmaceutical composition that "attenuates the effect of pathogenic bacterial enterotoxins," and a corresponding method comprising administering a pharmaceutical composition to a host in an amount "effective to attenuate the effect of the pathogenic bacterial enterotoxins." This amendment renders some of the Office's comments moot.

A. The Working Examples in the Specification Correlate to the Claimed Attenuation of Enterotoxin Effects and Polymorphonuclear Leukocyte Migration

Enablement of a pharmaceutical method and its corresponding composition does not require actual demonstration of the claimed effect *in vivo*, but only a reasonable correlation between the experiments described in the specification and the claimed *in vivo* effect. M.P.E.P. § 2164.06(a)(III) and § 2107.03. The M.P.E.P. points out that,

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when analyzing the sufficiency of working examples, "if the art is such that a particular model is recognized as correlating to a specific condition, then it should be accepted as correlating unless the examiner has evidence that the model does not correlate." M.P.E.P. § 2164.02. Moreover, the correlation need only be *reasonable*, not rigorous or exact. M.P.E.P. § 2107.03(I); *Cross v. Iizuka*, 224 U.S.P.Q. 739 (Fed. Cir. 1985).

Here, the specification presents data that reasonably correlates to the claimed subject matter. *See* M.P.E.P. § 2164.02 and §§ 2107-2107.03. Claims 1 and 10 recite a pharmaceutical composition and method that "attenuates the effect of pathogenic bacterial enterotoxins." (See the specification at page 12, lines 5-22.) The specification demonstrates this attenuation of bacterial enterotoxins, using cadaverine as the diaminoalkyl compound, in a series of experiments presented in Examples 2-7. The assays depicted in those examples are classic, routine tests of bacterial pathogenicity and are known to correlate with pathogenicity *in vivo*.

For instance, Example 5 describes a rabbit ileal loop assay showing that the *cadA* gene, which produces a lysine decarboxylase enzyme that synthesizes cadaverine, blocks the activity of *Shigella* enterotoxins. (Specification at page 30, lines 2-16.) The ileal loop assay is described in Sansonetti et al., (*Infect. Immun.*, 39: 1392-1402 (1983); of record), for example. It is an *in vivo* assay in which enterotoxin activity is measured by the ability of the toxin to cause secretion of fluid in rabbit intestinal loops. (See the specification at page 24, lines 3-14, for a description of the assay.) Fluid secretion into the gut is a hallmark of many gastrointestinal bacterial infections, leading to the potentially lethal diarrhea and dehydration observed in patients suffering from those infections. Using the ileal loop assay, Applicants obtained the novel finding

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that expression of *cadA*, and thus synthesis of cadaverine, was both necessary and sufficient to prevent *Shigella* from causing such fluid secretions.

Applicants used the Ussing chamber assay in further experiments because it can precisely measure the activity of enterotoxins responsible for the fluid secretion observed in the rabbit ileal loop assay. (Specification at page 30, line 17, to page 37, Table 5; Examples 6 and 7.) The assay protocol is described at page 24, line 15, to page 25, line 12, of the specification, as well as by Fasano et al. (*Proc. Natl. Acad. Sci., USA*, 88: 5242-6 (1991), of record). Table 3, at page 34, shows that the activity of *Shigella* enterotoxins is inhibited in a dose-dependent manner by micromolar concentrations of cadaverine. Tables 4 and 5, at pages 36 and 37, show that micromolar concentrations of cadaverine inhibit the activity of enterotoxins from a number of bacterial pathogens, such as *Shigella flexneri*, *Shigella dysenteriae*, *Yersinia enterocolitica*, *Bacterioides fragilis*, and *Campylobacter jejuni*. Therefore, the rabbit ileal loop and Ussing chamber assay results correlate to the claimed compositions and methods by showing that cadaverine reduces enterotoxin activity.

Example 8 shows that cadaverine is also able to block *Shigella*-induced transepithelial migration of polymorphonuclear (PMN) leukocytes, which can lead to inflammatory reactions. (Specification at page 25, lines 13-19, and at pages 38-46.) The T84 model intestinal epithelium used for this experiment is described, for example, in several references dated from 1975 to 1992, listed at page 38, lines 3-6, of the specification. Thus, it is a known model of transepithelial migration *in vivo*. Using this model, Applicants first observed that treatment of the baso-lateral surface of the epithelium with three micromolar concentrations of cadaverine led to a dose-dependent

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reduction in PMN migration across the epithelium, as shown in Figure 3. This result is further described at page 42, lines 1-16. Second, Applicants observed that cadaverine pre-treatment of the pathogenic bacteria, or cadaverine treatment of an epithelium already infected with *Shigella*, also blocked PMN migration. (Specification at pages 44-46, Example 8 parts C and D.) These results correlate to the subject matter of claims 9 and 14, for example.

B. The Results Demonstrated for Cadaverine Correlate to those Expected for other Claimed Diaminoalkyl Compounds

The Office contends that "success of one diaminoalkyl group . . . does not directly correlate to a different diaminoalkyl compound's success in the same method." (Office Action at page 3.) One of ordinary skill in the art, however, would find the experimental results with cadaverine, described above, as representative of those with other diaminoalkyl compounds because of the way in which Applicants discovered cadaverine's effects and because of the known chemical and functional similarities of these compounds.

Cadaverine is produced by the *Shigella* lysine decarboxylase enzyme, which is expressed from the *cadA* gene. Applicants discovered that expressing the *cadA* gene in infectious *Shigella* strains is alone sufficient to turn a previously highly pathogenic bacteria into a non-pathogenic one. (Specification at page 10, line 12, to page 11, line 2.) Further, cadaverine alone is both necessary and sufficient to inhibit enterotoxin activity according to the claims. (Specification at page 12, lines 5-22.)

Other bacteria are known in the art to possess similar amino acid decarboxylase genes that make diaminoalkyl compounds such as agmatine, putrescine and spermidine instead of cadaverine. (Specification at page 10, lines 1-11; *See also* Dela

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Vega & Delcour, *J. Bacteriology* 178(13): 3715-21 (1996), at page 3715, bottom of first column, of record.) Based on this knowledge, it is reasonable to expect from Applicants' findings that these other amino acid decarboxylase genes and other diaminoalkyl compounds might serve the same anti-virulence purpose *in vivo* as *cadA* and cadaverine do.

Moreover, it would not require undue experimentation to determine if a given diaminoalkyl compound attenuates the activity of bacterial enterotoxins as guided by the specification. Indeed, as described previously, the rabbit ileal loop and Ussing chamber assays are well known in the art and are routine tests of bacterial pathogenicity. Therefore, they may be used for screening a variety of compounds without undue experimentation.

Finally, the Office supports its comments regarding the predictability of different diaminoalkyl compounds by citing Wang (U.S. Patent No. 5,502,055). (Office Action at page 3; contending that putrescine is effective against endotoxic shock, but spermine is not effective.) However, Wang is not relevant to these claims. Wang discusses endotoxic shock caused by an *endotoxin*. The present claims relate to attenuating the effects of *enterotoxins*. The two types of toxins have completely different modes of action. Endotoxins produce antigenic peptides that cause an inflammatory response in the host, while enterotoxins function, for example, to mediate the diahrreal response. (See Exhibit A for a discussion of the two types of toxins.)

C. Determination of an Effective Concentration *In Vivo* Does Not Require Undue Experimentation

The Office also contends that this application presents no *in vivo* treatment experiments. (Office Action at page 3.) Yet, *in vivo* tests related to this invention are, in

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fact, presented in this application in the rabbit ileal loop assays, and are well within the level of ordinary skill in the art. Enablement depends upon whether the necessary experimentation is "undue," not solely on its amount or complexity. M.P.E.P. § 2164.06. Indeed, even a considerable amount of experimentation is permissible, if it is routinely performed by those of ordinary skill in the art, and if the specification, in conjunction with the knowledge available to those in the art, provides sufficient guidance as to how to perform it. M.P.E.P. § 2164.06; *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988).

The Office finally contends that diaminoalkyl compounds may be toxic under certain conditions and asserts that Applicants' specification does not provide guidance as to how to use the compounds such that they will not cause toxic side-effects. (Office Action at page 4.) The Office cites three abstracts by Sanz et al., Ordoñez et al., and Gabastou et al., to support this contention. Full copies of each of these articles are provided in the attached Information Disclosure Statement.

Before discussing each of these articles, Applicants first point out that toxicity is one of the factors considered in Phase I FDA "safety and efficacy" testing, which is beyond the level of the enablement inquiry. M.P.E.P. 2107.03(V); *In re Brana*, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995). Instead, the enablement inquiry is based upon the standard of credible utility. *Id.* Second, diaminoalkyl compounds, as the present specification demonstrates, are naturally produced by commensal bacteria such as *E. coli* in the gastrointestinal tract of their hosts. As the articles by Sanz and Ordoñez point out, such compounds are also commonly found in fermented food products and in meats. Third, many pathogenic bacteria, particularly those that lead to diseases like cholera or dysentery, produce acute diseases that may be imminently life-threatening,

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making the potential side-effects of treatments less important than for chronic, non-life-threatening diseases.

Applicants submit that the Sanz, Ordoñez, and Gabastou articles do not demonstrate that diaminoalkyl compounds are toxic to humans. For example, the Sanz article does not provide any data that appears relevant to the effects of diaminoalkyl compounds on human health. Instead, it addresses whether amine-containing compounds affect aminopeptidase enzymes produced by *Lactobacillus sake* bacteria, and thereby alter the taste or ripening conditions of fermented foods. (Sanz at page 870, Introduction, second paragraph spanning cols. 1 and 2.) Thus, Sanz is concerned with improving fermentation processes, not with identifying toxic compounds. The effect of polyamines on bacterial aminopeptidase enzymes is irrelevant in the context of the instant invention. Indeed, Sanz does not provide any evidence that altering the activity of an enzyme produced by fermentation bacteria has any effect on human health, or that the *Lactocillus sake* bacteria plays any role in human health. Further, Sanz points out that amines are normally present in ingested food such as meats at up to 1 mM concentration without causing any significant health effects. (Sanz at page 872, second to last paragraph.)

Ordoñez and co-authors also studied the concentrations of certain amine-containing compounds during the fermentation of foods (in this case ewe's milk cheese). The introduction to the article recites that histamine and tyramine can cause food poisoning. (Ordoñez at page 1371, paragraph bridging cols. 1 and 2.) But neither of these compounds are "diaminoalkyl compounds," as Applicants claim. Instead, they are monoamines. Moreover, the article presents no data related to the toxicity of any

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amine, but simply reports the levels of several amines in fermented cheese. As to putrescine and cadaverine, the article merely states that these other amines "may" increase the already toxic effects caused by high levels of histamine. (Ordoñez at page 1371, col. 2.) But, histamine-induced food poisoning is not relevant to this invention, which instead concerns pathogenic bacterial enterotoxins.

While the Gabastou article is in French, the English-language Summary provides a synopsis of its teachings, and Applicants provide additional comments about the article here. This article concerns only a single patient, called "Patrick C.," who was suffering from a psychiatric disease at the time of the study. (Gabastou at page 276, col. 2., bottom.) The authors assessed the stool concentrations of histamine, tyramine, cadaverine, and putrescine, in the patient and three control subjects, to determine if there was any correlation between amine levels and the patient's psychotic symptoms. (See the English-language Summary and Table 1.) The results showed that the stool concentrations of the amines were level over time in the control subjects but varied in the patient, according to his psychotic "crises." (Gabastou at Fig. 1.) These results do not demonstrate that diaminoalkyl compounds have any toxic properties in normal subjects. Moreover, Gabastou presents results from only a single diseased patient. One of ordinary skill in the art would not conclude, based on data from only one individual, that humans suffering from central nervous system or psychotic disorders would find diaminoalkyl compounds to be harmful.

As further evidence that the claimed "diaminoalkyl compounds" may be administered as claimed without undue experimentation, Applicants note that the Dela Vega & Delcour *Journal of Bacteriology* article discussed in a following section of these

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remarks points out that these compounds are non-toxic to cells at concentrations about 300 to 1000-fold higher than those used in Applicants' assays. (80 mM for putrescine and 100 mM for cadaverine; Dela Vega & Delcour, *J. Bacteriology* vol. 178 at page 3719, bottom of column 1.) Finally, the United States patent by Wang (No. 5,502,055), upon which the Office relies at page 3 of the Office Action, suggests that toxicity is not an important factor in using putrescine to treat shock. Wang's specification does not discuss any potential toxicity of putrescine, though the Gabastou article that the Office cites in relation to putrescine toxicity was published prior to Wang's filing date.

In summary, the claims are enabled and the specification provides appropriate guidance to use the claimed compositions and methods throughout their full scope. Thus, Applicants respectfully request the withdrawal of this rejection.

IV. Claim Rejections under 35 U.S.C. § 102

The Office presents four separate rejections under § 102(b). Before discussing each rejection in detail, Applicants note that a single reference may only anticipate a claim if it teaches each and every element of that claim either expressly or inherently, and with as complete detail as in the claim itself. M.P.E.P. § 2131. In addition, in order to present a *prima facie* case of anticipation, the Office must support its conclusions with substantial evidence or scientific reasoning firmly grounded in fact. *In re Lee*, 61 U.S.P.Q.2d 1430 (Fed. Cir. 2002); *In re Zurko*, 59 U.S.P.Q.2d 1693 (Fed. Cir. 2001). Applicants respectfully traverse each of the following anticipation rejections because the references do not teach all of the claim elements and because the Office has not established that they do so by the required evidence or reasoning.

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A. Rejection of Claims 1 and 3-5 over Wang (U.S. Patent No. 5,502,055)

The Office rejects claims 1 and 3-5, alleging that they are anticipated by Wang. (Office Action at page 7.) Yet, Wang does not teach a diaminoalkyl compound (putrescine) and a pharmaceutically acceptable carrier in a composition or method wherein putrescine is in an amount effective to attenuate the activity of bacterial enterotoxins, as claimed. Instead, Wang relates to treatment of endotoxic shock. Applicants have informed the undersigned that, contrary to the Office's supposition, endotoxic shock is not a "gastrointestinal disorder" in accordance with this invention. Further, endotoxic shock is due to an *endotoxin*, such as lipopolysaccharide, and not to an *enterotoxin*, as claimed here. As explained above, these toxins have completely different functions. (See Exhibit A.) Indeed, in Wang, mice were exposed solely to the endotoxin lipopolysaccharide in a buffer solution, rather than to bacteria. (See Wang at col. 2, lines 6-11. Mice were exposed to *E. coli* lipopolysaccharide purchased from Sigma and dissolved in phosphate-buffered saline, not to *E. coli* bacteria.) Therefore, no enterotoxins were or could have been present. Nor does the Office claim that Wang's composition could have inherently included an effective amount of putrescine to attenuate the activity of an enterotoxin. For these reasons, Wang does not anticipate Applicants claimed invention, and Applicants request the withdrawal of this rejection.

B. Rejection of Claims 1, 3, and 4 over Keusch et al. (*Biochem. Biophys. Res. Comm.* 121(1): 69-76 (1984)).

The Office asserts that claims 1, 3, and 4 are anticipated by Keusch et al. (Office Action at pages 7-8.)

However, Keusch et al. does not anticipate Applicants' claims because, like Wang, it does not address enterotoxins. Nor are enterotoxins present in Keusch's *in*

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vitro experiments. Instead, Keutch et al. addresses Shiga toxin, a toxin produced by *Shigella disenteriae* 1. Applicants have informed the undersigned that enterotoxins are generally distinct from Shiga toxin in their structure, mode of action, and distribution within *Shigella* species. Shiga toxin is a protein synthesis inhibitor, while enterotoxins function, for example, to mediate the diahrreal response. (See Exhibit A and Exhibit B at page 7, for a discussion of different types of bacterial toxins.)

Further, Applicants' claims are structurally different from the putrescine solutions of Keusch et al., which were used solely for *in vitro* experiments. According to Applicants' claims, the putrescine solution must be in a "pharmaceutically acceptable carrier;" in other words, a carrier that is able to be directly administered to a patient such that the patient will not experience an adverse reaction or immunological response. Keutch et al. tested most of the compounds in McCoy's modified medium (MM), which, though it is a tissue culture medium, is "not intended for human or therapeutic use." (See Exhibit C, excerpt from JRH Biosciences catalog; Keusch et al. at page 70, first full paragraph.) In addition, putrescine was ineffective unless it was used in a solution containing 1% dimethylsulfoxide (DMSO). (Keusch et al. at page 70, line 5, and at page 71, lines 4-5.) DMSO is a cryoprotectant that increases the permeability of cells such that they can take up other compounds. (See information from Sigma-Aldrich and the Howard Hughes Medical Institute, Exhibit D.) DMSO is not necessarily a pharmaceutically acceptable substance and could be harmful if ingested. (*Id.*)

In summary, in addition to the fact that Keusch does not teach an "enterotoxin," it also does not teach a "pharmaceutically acceptable carrier." Thus, Applicants respectfully request the withdrawal of this rejection.

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C. Rejection of Claims 1, 2, and 4 over Dela Vega & Delcour (*EMBO J.* 14(23): 6059-65 (1995)) and Rejection of Claims 1-4 over Dela Vega & Delcour (*J. Bacteriol.* 178(13): 3715-21 (1996))

The Office rejects claims 1, 2, and 4 over Dela Vega & Delcour's publication in *EMBO Journal* ("Dela Vega 1995"), asserting that this publication teaches the use of cadaverine compositions as claimed. (Office Action at pages 8-9.) The Office also rejects claim 1-4 over Dela Vega & Delcour's article in the *Journal of Bacteriology* ("Dela Vega 1996"), asserting that it teaches putrescine and cadaverine compositions that meet the claimed limitations. (Office Action at pages 9-10.)

However, as with Keusch et al., discussed above, the two Dela Vega articles do not teach compositions of diaminoalkyls that are in a "pharmaceutically acceptable carrier," as defined above, such that the diaminoalkyl is able to "attenuate the activity of pathogenic bacterial enterotoxins." The Office has not set forth any substantial evidence as to how Dela Vega's solutions, used for patch-clamp assays and other *in vitro* experiments, are also "pharmaceutically acceptable" such that they could be immediately administered to a patient without adverse effects. Moreover, Dela Vega's articles discuss the effects of the diaminoalkyls on bacterial outer-membrane porin proteins. In contrast, the present invention relates to a completely different biological process: that of protecting animal cells from bacterial enterotoxins. Therefore, Applicants respectfully request the withdrawal of this rejection.

V. Conclusion

In view of the foregoing amendments and remarks, Applicants respectfully request the reconsideration and reexamination of this application and the timely allowance of the pending claims.

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Respectfully submitted,

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